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# A perspective on underwater photosynthesis in submerged terrestrial wetland plants

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## Abstract

**Background and aims** Wetland plants inhabit flood-prone areas and therefore can experience episodes of complete submergence. Submergence impedes exchange of $O_2$ and $CO_2$ between leaves and the environment, and light availability is also reduced. The present review examines limitations to underwater net photosynthesis ($P_N$) by terrestrial (i.e. usually emergent) wetland plants, as compared with submerged aquatic plants, with focus on leaf traits for enhanced $CO_2$ acquisition.

**Scope** Floodwaters are variable in dissolved $O_2$, $CO_2$, light and temperature, and these parameters influence underwater $P_N$ and the growth and survival of submerged plants. Aquatic species possess morphological and anatomical leaf traits that reduce diffusion limitations to $CO_2$ uptake and thus aid $P_N$ under water. Many aquatic plants also have carbon-concentrating mechanisms to increase $CO_2$ at Rubisco. Terrestrial wetland plants generally lack the numerous beneficial leaf traits possessed by aquatic plants, so submergence markedly reduces $P_N$. Some terrestrial species, however, produce new leaves with a thinner cuticle and higher specific leaf area, whereas others have leaves with hydrophobic surfaces so that gas films are retained when submerged; both improve $CO_2$ entry.

**Conclusions** Submergence inhibits $P_N$ by terrestrial wetland plants, but less so in species that produce new leaves under water or in those with leaf gas films. Leaves with a thinner cuticle, or those with gas films, have improved gas diffusion with floodwaters, so that underwater $P_N$ is enhanced. Underwater $P_N$ provides sugars and $O_2$ to submerged plants. Floodwaters often contain dissolved $CO_2$ above levels in equilibrium with air, enabling at least some $P_N$ by terrestrial species when submerged, although rates remain well below those in air.

## Introduction

Emergent wetland plants are well adapted to waterlogged soils, but can also experience episodes of complete submergence. Complete submergence has an impact on wild species in coastal marshes and river floodplains (Armstrong *et al.* 1985), and many rice crops are grown in regions threatened by floods, causing submergence (Jackson and Ram 2003).

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Complete submergence impedes the exchange of $O_2$ and $CO_2$ between leaves and the environment (Mommer and Visser 2005; Voesenek *et al.* 2006). Light availability to submerged plants also decreases, and markedly so when floodwaters are turbid (Mommer and Visser 2005; Voesenek *et al.* 2006). Restricted photosynthesis, but ongoing substrate consumption in respiration or fermentation, causes sugars to become depleted in submerged plants, which in turn can result in damage or even death from substrate exhaustion (Bailey-Serres and Voesenek 2008; Colmer and Voesenek 2009).

The interface between land and water is not well defined as water tables fluctuate with precipitation and evaporation, so that plants experience variable periods and depths of flooding (Sculthorpe 1967). Plants exploit niches across these dynamic flooding gradients, but functional classification of plant types lacks sharp boundaries owing to the continuum of diversity. Notwithstanding these difficulties, plants from the wettest end of the gradient have been classified into two main groups: (i) aquatic plants that primarily live completely submerged and (ii) amphibious plants that live with emergent shoots or develop water forms when submerged (Iversen 1936; cited by Sculthorpe 1967). Emergent wetland plants typically maintain a large portion of their shoots in air, but occasionally become completely submerged. To clearly distinguish these emergent plants from other wetland species with shoot portions in air (e.g. emergent amphibious plants), we refer to this functional group as 'terrestrial wetland plants' (present review; Colmer and Pedersen 2008; Pedersen *et al.* 2010). Such distinction is important as terrestrial wetland plants typically grow vigorously in waterlogged soils and/or areas with shallow standing water, with the depth limit being determined by capacity for transport of atmospheric $O_2$ to belowground tissues (Sorrell *et al.* 2000).

For submerged terrestrial plants, $O_2$ deficiency and escape responses via shoot elongation have been elucidated, revealing sophisticated signalling, changes in gene expression and altered metabolism during submergence (e.g. reviewed by Bailey-Serres and Voesenek 2008, 2010). The capacity for some net photosynthesis ($P_N$) to continue when under water enhances plant tolerance of submergence, as $P_N$ provides $O_2$ for internal aeration and sugars for energy metabolism and growth (Mommer and Visser 2005).

The present review examines limitations to underwater $P_N$ by terrestrial wetland plants and compares their functioning with aquatic plants. Our focus here on underwater $P_N$ as related to the ecophysiology of submergence tolerance adds to the vast knowledge on root adaptations in wetland species. Roots of wetland plants typically contain large volumes of aerenchyma, often a barrier to radial $O_2$ loss, and the ability to tolerate tissue $O_2$ deficits and reduced phytotoxins in waterlogged soils (Armstrong 1979; Jackson and Armstrong 1999; Bailey-Serres and Voesenek 2008; Colmer and Voesenek 2009). Here, we show that underwater $P_N$ by submerged terrestrial wetland plants is limited by $CO_2$ availability even though floodwaters commonly contain dissolved $CO_2$ above air equilibrium, and so leaf traits influencing underwater $P_N$ are important for submergence tolerance.

## The submergence environment during overland floods

Floods differ in seasonal timing, duration, depth and frequency (e.g. Vervuren *et al.* 2003). Floodwater properties (e.g. water turbidity and dissolved $CO_2$) that influence plant functioning can also differ substantially; light and $CO_2$ available to submerged plants determine underwater $P_N$ and survival (Mommer and Visser 2005; Pedersen *et al.* 2010). Thus, the flooding regime and water properties influence plant species distributions in flood-prone areas (Armstrong *et al.* 1985; Voesenek *et al.* 2004). In this section, we discuss three types of flooding events that can affect terrestrial wetland plants: flash floods, seasonal floods and tidal flooding.

Flash floods occur when heavy rainfall causes water levels to rise rapidly for a variable period of time, especially as run-off moves to low-lying areas (Setter *et al.* 1987; Brammer 1990; Ram *et al.* 1999). Flash floods in some regions can be more likely to occur during specific seasons, but in other areas flash flooding is not season specific. Seasonal floods are caused by an increase in water flow that surpasses the capacity of rivers in a landscape to discharge the large volumes of water, resulting in overflow of banks and floodplains. The origin of the increased water flow can be seasonal precipitation and/or snow melt (Brammer 1990). Tidal flooding impacts coastal plains and estuarine marshes with depths determined by the moon's cycle (e.g. neap tides and spring tides). Tidal floods involve saline water, whereas overland floods are usually freshwater, with the exception of some inland catchments with salt lakes.

Flooding can occur with various combinations of chemical and physical properties in the water; $O_2$, $CO_2$, temperature, pH and light can all vary (Setter *et al.* 1987; Pérez-Lloréns *et al.* 2004). Seawater pH is well buffered as it contains $HCO_3^-$ (2.2 mM; Millero *et al.* 1998) and $HCO_3^-$ also buffers against severe depletion of dissolved $CO_2$. In freshwater floods, $HCO_3^-$ and $CO_2$ concentrations are highly variable, but dissolved $CO_2$ is commonly above air equilibrium (Table 1). The high $CO_2$ concentrations typically result from respiration by

**Table 1 Dissolved $CO_2$ and $O_2$ concentrations in various types of floodwaters.** Medians with ranges in parentheses.

| Environment | $CO_2$ (μM) | $O_2$ (μM) |
|---|---|---|
| Terrestrial | | |
| Flash flood [1,2] ($n = 4$) | 1040 (3–1953) | 150 ('0'–280) |
| Seasonal flood [3–6] ($n = 6$) | 365 (47–1600) | 79 ('0'–240) |
| Tidal flood [7,8] ($n = 4$) | 16 (3–49) | 292 (188–522) |
| Aquatic | | |
| Streams and rivers [9,10] ($n = 31$) | 133 (11–836) | n.a. |
| Ponds (< 1 ha) [11] ($n = 7$) | 59 (<1–374) | n.a. |
| Lakes [11] ($n = 17$) | 45 (11–210) | n.a. |

[1,2](Ram et al. 1999; Setter et al. 1987); [3–6](Hamilton et al. 1997; Hamilton et al. 1995; Richey et al. 2002; Valett et al. 2005); [7,8](Pérez-Lloréns et al. 2004; Winkel et al. 2011); [9](Sand-Jensen and Frost-Christensen 1998); [10](Jonsson et al. 2003) [11](Staehr et al. 2011).
n.a., not available.
$O_2$ was not measured in the water surveys conducted in 9, 10 and 11.

organisms consuming labile carbon compounds (i.e. a net heterotrophic system); in addition, some water bodies receive $CO_2$-enriched groundwater stream flows. By contrast, in net autotrophic systems photosynthesis depletes $CO_2$ and produces $O_2$. So, $O_2$ concentrations in floodwaters can range from severely hypoxic (net heterotrophic) to well above air equilibrium (net autotrophic).

Temperature during flooding events can also vary widely (e.g. ~6–37 °C; Hamilton et al. 1997; Valett et al. 2005; Pedersen et al. 2011a), depending on location and season. Respiration increases at warmer temperatures, which can deplete $O_2$, and $O_2$ concentration is further reduced owing to lower $O_2$ solubility in water as temperature increases. So, the imbalance between $O_2$ demand and supply to submerged terrestrial plants can be further exacerbated as temperature increases.

Flow rates during floods have only been reported, to our knowledge, in three papers: data are available for two flash floods and one seasonal river flood, and flows ranged from 0.002 to 0.3 m $s^{-1}$. Flow rates affect the thickness of diffusive boundary layers (DBLs) and thereby influence gas and nutrient exchanges with submerged plants (Binzer et al. 2005; Pedersen et al. 2009). So, underwater $P_N$ can increase with increasing flow velocity since the DBLs become thinner (Jones et al. 2000), but the response would plateau (cf. $O_2$ supply; Binzer et al. 2005) or even decline again if flows cause excessive shoot agitation (Madsen et al. 1993a).

Light regimes in floodwaters are dependent on several factors. When floodwaters contain suspended particles or dissolved coloured organic matter (e.g. tannins in Amazonian floodwaters; Parolin 2009), light availability will be reduced. Particle suspension can be highest during early stages of floods and particles often then settle; however, if particles settle on leaves these can still limit light. Waters of high nutrient availability typically support growth of microalgae, with dense populations of both biofilms and phytoplankton leading to lower light penetration to leaf surfaces (Sand-Jensen and Sondergaard 1981; Sand-Jensen and Borum 1991; Lassen et al. 1997) and consequently also shallower depth limits for plant colonization (Sand-Jensen 1990). Examples of light reductions are available for floodwaters in the rice fields of India and Thailand; the depth at which 50% light remained varied from 0.07 to 0.7 m (Setter et al. 1987; Ram et al. 1999).

How does the submerged environment experienced by terrestrial wetland plants compare with that of water bodies containing permanent aquatic vegetation? In brief, environments supporting healthy stands of submerged aquatic plants, such as the shallow sea, and areas within rivers and lakes, also share many of the above-mentioned constraints to plant growth. Light attenuation in the water column (caused by water itself, dissolved coloured organic matter, phytoplankton and other particles) determines the maximum depth of colonization by aquatic plants. Seagrasses typically grow down to ~10% of the surface light (Duarte 1991), whereas the depth penetration of plants in freshwater lakes is down to <1% and typically ~5% of the surface light (Canfield et al. 1985). The lower light compensation points for the growth of deep-colonizing freshwater plants result from these having higher shoot-to-root ratios than seagrasses. The deepest-growing freshwater plants, such as species of *Ceratophyllum* and *Utricularia*, do not produce roots at all (Cook 1990). Similar to terrestrial floodwaters, dissolved inorganic carbon (DIC) in freshwater can also vary widely (e.g. from 0.02 to 5.6 mM in British lakes; Maberly and Spence 1983). Depending on pH, the above DIC concentrations may result in dissolved $CO_2$ levels from near or below air equilibrium (15 μM in freshwater at 20° C) to waters in streams/rivers, ponds and lakes that are typically supersaturated (Table 1); ponds can even contain up to 2000 μM $CO_2$ (133-fold air equilibrium). The temperature in most water bodies fluctuates significantly less than surrounding air due to the much higher specific heat capacity of water compared with air (Hutchinson 1957), but there are exceptions, such as in shallow rock pools with large diel fluctuations (Pedersen et al. 2011a). Finally, the flow velocity in aquatic environments also varies widely, as described earlier for terrestrial floods, from almost stagnant conditions in ponds and deeper areas of lakes to very high velocities in rivers and in surf zones of the sea (2–3 m $s^{-1}$; Vogel 1994). In fast-flowing water or in wave-zones, the strap-shaped leaves typical of some aquatic plants are highly adaptive as this morphology reduces the pressure drag (Vogel 1994).

In summary, floodwaters faced by terrestrial plants invoke some common constraints of restricted gas exchange and lower light availability, but conditions ($O_2$, $CO_2$, light and temperature) differ between locations and times, posing variable challenges to plant functioning during submergence. Floodwater chemical and physical properties, in addition to the well-recognized importance of seasonal timing, duration, depth and frequency of floods (e.g. Vervuren *et al.* 2003), will influence plant growth and survival during submergence.

## Net photosynthesis under water

Low $CO_2$ and/or low light can restrict $P_N$ by submerged plants (Sand-Jensen 1989). This review focuses on $CO_2$ acquisition. Aquatic species possess leaf traits to enhance DIC supply and thus rates of underwater $P_N$. In Table 2, we compare the leaf traits of terrestrial wetland plants with those of submerged aquatic plants. Below we (i) summarize knowledge of morphological and anatomical leaf traits, and photosynthetic pathways including carbon-concentrating mechanisms (CCMs), and (ii) compare the rates of underwater $P_N$ by different types of aquatic and terrestrial wetland plants, as influenced by these leaf traits.

### Leaf traits of terrestrial wetland plants and submerged aquatic plants

Leaf morphology determines boundary layer resistances to exchange of dissolved gases and ions (Madsen and Sand-Jensen 1991). Boundary layer resistance can limit the rates of $CO_2$ uptake and thus reduce underwater $P_N$ in submerged plants as diffusion is $10^4$-fold slower in water than in air (Vogel 1994). Morphological traits (Table 2) that reduce the DBL resistance, by decreasing the distance to the 'leading edge' (Vogel 1994), include leaf shapes of small, dissected/lobed and/or strap-like leaves. In addition, aquatic leaves lack trichomes, thus avoiding the development of thicker boundary layers adjacent to their surfaces. Leaves of aquatic species also tend to be thin (Table 2), although there are several exceptions (e.g. isoetids; Sand-Jensen and Prahl 1982). Thin leaves have short internal diffusion path lengths, reducing the overall resistance for $CO_2$ to reach chloroplasts (Madsen and Sand-Jensen 1991; Maberly and Madsen 2002). One example is the lamina of *Najas flexilis*, which is only two cell layers (Tomlinson 1982). In cases where leaves are relatively thick, $CO_2$ is typically sourced from sediments (e.g. isoetids; Winkel and Borum 2009), and these leaves tend to be of high porosity to facilitate internal gas phase diffusion (Pedersen and Sand-Jensen 1992; Pedersen *et al.* 1995; Sand-Jensen *et al.* 2005).

**Table 2 Comparison of leaf traits influencing gas exchange and photosynthesis by terrestrial wetland plants when under water and by submerged aquatic plants.** Modified from Sculthorpe (1967) with data from additional references as indicated by superscripts: [1](Neinhuis and Barthlott 1997), [2](Colmer and Pedersen 2008), [3](Maberly and Madsen 2002).

| Leaf traits | Terrestrial wetland plants | Submerged aquatic plants |
|---|---|---|
| Morphology | | |
| Leaf size | Medium–large | Small–medium |
| Dissected/lobed | Rare | Common |
| Strap-shaped | Rare | Common |
| Leaf thickness[a] | Moderate–thick | Thin |
| Surface hydrophobicity/leaf gas films[1,2] | Common | Absent |
| Hairs/trichomes | Rare | Absent |
| Anatomy | | |
| Stomata | Always present | Absent/non-functional |
| Cuticle | Always present | Absent/highly reduced |
| Chloroplasts in epidermal cells | Only in guard cells | Common |
| Aerenchyma | Variable | Variable |
| Porosity of lamina | High in thick, low in thin, lamina | High in thick, low in thin, lamina |
| Supporting fibres | Always present | Rare |
| Photosynthetic pathway/CCM[3] | | |
| C3 | Common | Common |
| C4 | Rare | Rare (but uncertain) |
| CAM | Absent | Rare |
| $HCO_3^-$ use | Absent | Common |

[a]For data on SLA see Fig. 2. Other leaf features/properties can also differ between terrestrial wetland plants and submerged aquatic plants, such as: venation, lignification, stiffness, surface topography, differences between adaxial and abaxial surfaces, and in the case of some halophytic wetland species, presence of salt bladders and glands.

In addition to these morphological traits, leaves of aquatic species also have anatomical traits that further reduce diffusive resistances for $CO_2$ to reach chloroplasts (Table 2). Aquatic leaves lack, or have very reduced, cuticles. Diffusion across the cuticle is the main pathway of dissolved gas exchange as the leaves lack stomata, or if present, the stomata are non-functional (Pedersen and Sand-Jensen 1992). The diffusion path length to chloroplasts is also minimized by having these organelles in all epidermal cells, and in sub-epidermal cells the chloroplasts are positioned towards the exterior (Table 2).

Submerged aquatic plants also display physiological adaptations to increase the $CO_2$ concentration at Rubisco, the site of carboxylation (Table 2)—these are referred to as CCMs (Maberly and Madsen 2002; Raven *et al.* 2008). In submerged aquatic plants, CCMs include $HCO_3^-$ use (Prins and Elzenga 1989), C4 (Magnin *et al.* 1997), C3–C4 intermediates (Keeley 1999) and Crassulacean Acid Metabolism (CAM) photosynthesis (Keeley 1998). Carbon-concentrating mechanisms increase $P_N$ in $CO_2$-limited submerged environments, and have also been suggested to diminish photorespiration (Maberly and Madsen 2002). Photorespiration results from the oxygenase activity of Rubisco and is promoted by a low $CO_2$:$O_2$ ratio (Ogren 1984), a condition common in leaves when under water (Bowes 1987). The low $CO_2$ availability in aquatic environments would in itself lower the $CO_2$:$O_2$ ratio. Moreover, $O_2$ in submerged leaves can be high as escape is slower than production in $P_N$; $O_2$ escape is not only hampered by DBLs but also by the relatively low $O_2$ solublity in water; $CO_2$ is 28.5-fold more soluble than $O_2$ at 20° C (Baranenko *et al.* 1990). Reduced photorespiration in a submerged aquatic CAM plant has been recently demonstrated (Pedersen *et al.* 2011*b*), supporting the view that CCMs do reduce photorespiration in aquatic species.

By contrast with aquatic species, leaves of terrestrial wetland plants lack most of the features described above (Table 2) and so suffer from large diffusion limitations to $CO_2$ supply for $P_N$ when under water, unless they possess leaf gas films (Raskin and Kende 1983; Colmer and Pedersen 2008; Pedersen *et al.* 2009) or produce submergence-acclimated leaves (Mommer and Visser 2005). Below, we evaluate underwater $P_N$ by leaves of terrestrial wetland plants and then consider the occurrence and functioning of leaf gas films.

## Net photosynthesis of aquatic and submerged terrestrial wetland plants; leaf traits enhance $CO_2$ supply

The most comprehensive comparison of underwater $P_N$ by aquatic and terrestrial wetland plants is the study by Sand-Jensen *et al.* (1992). Thirty-five species of four life forms (terrestrial, amphibious homophyllous, amphibious heterophyllous and aquatic species) were compared (listed in Appendix 1). Inclusion of data from other studies in the present analysis was constrained by differences in techniques and conditions used for underwater $P_N$ measurements, e.g. $CO_2$, temperature and light (Appendix 2).

Classifications of wetland plants into functional groups are convenient, but are also imperfect as the boundaries are not sharp (see Introduction). As examples, some terrestrial wetland species produce new leaves when submerged and these can display some acclimation to the underwater environment (Mommer *et al.* 2007). Similarly, homophyllous amphibious plants can also display some acclimation, e.g. thinner cuticles and modestly thinner leaves when formed under water (Nielsen 1993), but these changes are far more subtle than those displayed by heterophyllous amphibious plants. Not surprisingly, different authors have classified some species into different life forms. Here, our focus is on the comparison of underwater $P_N$ of leaves formed (i) in air by terrestrial wetland species, (ii) under water by amphibious homophyllous species, (iii) under water by amphibious heterophyllous species and (iv) under water by aquatic species.

An additional noteworthy feature of the study by Sand-Jensen *et al.* (1992) was documentation of dissolved $CO_2$ levels in lowland stream habitats. Underwater $P_N$ was measured at ambient and at elevated $CO_2$ concentrations, to provide rates of relevance to the field situation as well as $CO_2$-saturated $P_N$ for aquatic leaf types. The level of elevated $CO_2$ used (~800 μM, being ~50-fold air equilibrium) would have saturated $P_N$ by the aquatic leaf types. It is uncertain whether rates were $CO_2$ saturated for some of the terrestrial leaf types, which can require as much as 75-fold of air equilibrium $CO_2$ when submerged (Colmer and Pedersen 2008).

We compare the rates on the dry mass basis (Fig. 1A and B) used by Sand-Jensen *et al.* (1992) and also on a projected leaf surface area basis (Fig. 1C and D); conversions used specific leaf area (SLA) data in the literature (Fig. 2; Appendix 1). Data for SLA were not available for three of the aquatic and three of the terrestrial wetland species in Sand-Jensen *et al.* (1992), so these six were omitted from the present analysis (Appendix 1).

The overall beneficial effects of aquatic leaf traits (Table 2) for underwater $P_N$, as well as the generally poor performance of leaves of terrestrial plants, were clearly demonstrated in Sand-Jensen *et al.* (1992). These authors highlighted that (i) underwater $P_N$ on a mass basis increased from terrestrial, then amphibious, to truly aquatic leaf types and (ii) Danish lowland

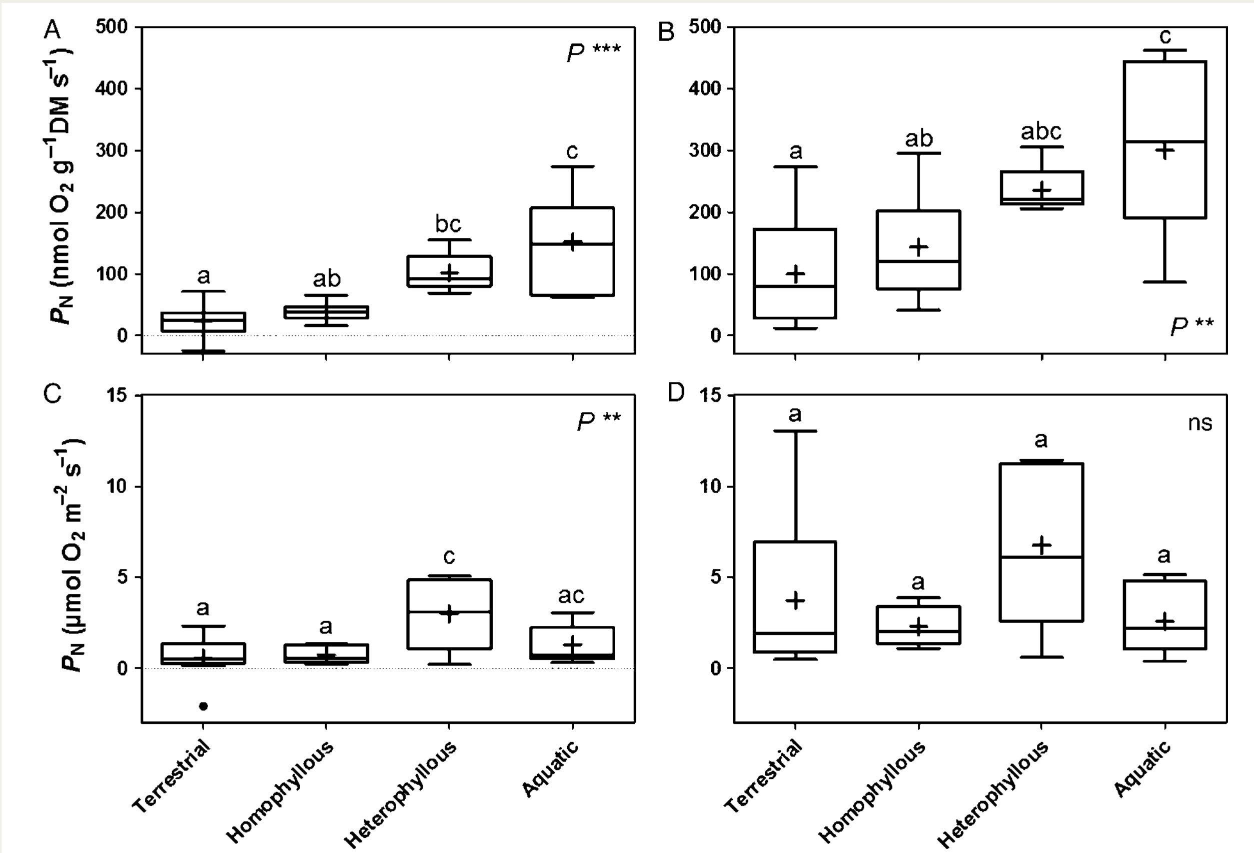


**Fig. 1 Underwater net photosynthesis ($P_N$) in terrestrial wetland plants, in amphibious homophyllous or heterophyllous wetland plants and in submerged aquatic plants.** Net photosynthesis was measured at 15° C and is expressed per leaf dry mass (A and B) or per projected leaf area (C and D) at ambient $CO_2$ levels (90–400 µM in the natural habitats; A and C) or at elevated $CO_2$ levels (800 µM; B and D). Species and SLA data sources are listed in Appendix 1. Our analysis focused on the study by Sand-Jensen *et al.* (1992) as it is the most comprehensive available; addition of other data was constrained by differences in techniques and conditions used (e.g. $CO_2$ and temperature; Appendix 2). Terrestrial, leaves formed in air by emergent wetland plants; homophyllous, leaves formed under water by amphibious wetland plants; heterophyllous, leaves formed under water by amphibious wetland plants; aquatic, leaves formed under water by submerged aquatic plants (cf. Sculthorpe 1967). Rates on a mass basis (A and B) were converted to an area basis (C and D) using the published SLA data (Fig. 2, Appendix 1). The box–whisker plot shows the median, 10 and 90 percentiles, minimum and maximum values, and means are shown as '+'; the dot in the terrestrial column indicates an outlier. Differences amongst means of the four plant groups within each panel were tested by one-way analysis of variance and Tukey's multiple comparison tests. ** $P < 0.01$ and *** $P < 0.001$. Means with the same letter within each panel do not differ significantly at the 95% confidence interval.

stream waters are commonly supersaturated with $CO_2$, allowing even some terrestrial species to have adequate $P_N$ for growth when submerged in these habitats.

The higher $P_N$ by aquatic leaf types per unit mass with near-ambient $CO_2$ concentrations (~90–400 µM) demonstrates the higher C-return per unit of dry mass investment by these leaf types in an underwater environment as compared with terrestrial types (Fig. 1A). When external $CO_2$ was supplied at an elevated level of ~800 µM (Fig. 1B), underwater $P_N$ values by the aquatic and heterophyllous amphibious leaves still exceeded those of the terrestrial and homophyllous leaf types. The low rates by terrestrial leaves even with elevated $CO_2$ further demonstrate the large diffusion limitations for $CO_2$ entry that restrict underwater $P_N$.

Expression of underwater $P_N$ rates on a surface area basis, the units typically used in terrestrial plant physiology (whereas in aquatic sciences, rates are typically expressed per unit dry mass), interestingly, removes differences between the terrestrial and aquatic leaf types, at both ambient and elevated $CO_2$ (Fig. 1C and D). The order of magnitude of higher SLA (Fig. 2) of aquatic and many amphibious leaf types clearly sets an upper

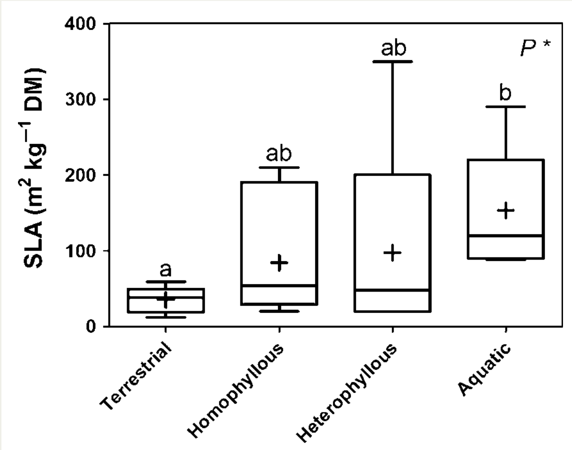


Fig. 2 **Specific leaf area in terrestrial wetland plants, in amphibious homophyllous or heterophyllous wetland plants, and in submerged aquatic plants.** The box-whisker plot shows the median, 10 and 90 percentiles, minimum and maximum values, and means are shown as '+'. Species and data sources are listed in Appendix 1. Differences amongst means of SLA of the four plant groups were tested by one-way analysis of variance and Tukey's multiple comparison tests. ** $P < 0.01$. Means with the same letter do not differ significantly at the 95% confidence interval.

limit for $P_N$ on an area basis. Maximum $P_N$, however, would rarely be achieved in most aquatic environments owing to light and $CO_2$ limitations (Sand-Jensen 1989; Kirk 1994) so that the lower $CO_2$-saturated rates of $P_N$ on an area basis for aquatic leaves would be of little consequence for their life under water.

Comparisons of the rates of underwater $P_N$ by terrestrial wetland plant leaf types with those achieved by aquatic leaf types are informative with respect to performance when submerged (Fig. 1), but here we also consider how these rates under water compare against those in air. For the terrestrial wetland species in Fig. 1, we could only find data on $P_N$ in air for three (*Carex elata*, *Ranunculus repens* and *Phragmites australis*; Appendix 1); $P_N$ in air at ambient $CO_2$ was 12.5–17 $\mu$mol $m^{-2}$ $s^{-1}$. When submerged with $CO_2$ at levels near ambient (but well above air equilibrium in these habitats), the mean $P_N$ under water was only 9% of that in air (cf. Fig. 1C). Thus, underwater $P_N$ is greatly reduced when terrestrial wetland species become submerged.

The analyses presented above for underwater $P_N$ by leaves of terrestrial wetland plants involved experiments in which leaves growing in air were tested under water. Several terrestrial wetland species produce new leaves when submerged, and these can display some acclimation to the underwater environment (e.g. thinner cuticles and thinner leaves; Mommer *et al.* 2007). Acclimated leaves have decreased resistances against $CO_2$ and $O_2$ movement across the cuticle and epidermis (Mommer and Visser 2005; Mommer *et al.* 2007). The best example is the several-fold reduction in cuticle resistance and thus the 69-fold higher underwater $P_N$ at an external $CO_2$ concentration of 250 $\mu$M by *Rumex palustris* (Mommer *et al.* 2006). Although a study of seven terrestrial wetland species established the formation of a thinner cuticle as a common response when submerged, and demonstrated enhanced underwater gas exchange, the degree of this response was not correlated with submergence tolerance among these species (Mommer *et al.* 2007). These anatomical, and in some cases morphological (e.g. *R. palustris* leaves are also more elongated), changes in submerged leaves of terrestrial species are much more subtle than the altered leaf development displayed by amphibious heterophyllous species which produce true aquatic leaf types when under water (Nielsen 1993).

In summary, $P_N$ by terrestrial wetland plants is reduced markedly when they are submerged. Leaves of terrestrial wetland plants generally lack the numerous beneficial leaf traits for underwater $P_N$ possessed by aquatic plants, although new leaves can display some acclimation (e.g. thinner cuticles and higher SLA). In addition, as discussed in the next section, some leaves of terrestrial wetland species retain a gas film when submerged, a trait that also enhances underwater $P_N$.

## Leaf gas films enhance the net photosynthesis of submerged terrestrial wetland plants

Many terrestrial wetland plants have water-repellent (i.e. hydrophobic) leaf surfaces, resulting in self-cleaning by water droplets as these run off leaves (Neinhuis and Barthlott 1997). Leaf water repellence has been assessed by measurement of water droplet contact angles with the surface (Adam 1963; Brewer and Smith 1997; Neinhuis and Barthlott 1997)—angles of 140° or more indicate a hydrophobic surface whereas angles of 110° or less indicate a wettable surface. Water repellence (i.e. surface hydrophobicity) is determined by the micro- and nano-structures of the surface, as well as wax crystals (Wagner *et al.* 2003; Bhushan and Jung 2006).

Superhydrophobic leaves retain a microlayer of gas when submerged, referred to as 'gas envelopes' (Setter *et al.* 1989) and/or 'leaf gas films' (Colmer and Pedersen 2008). We prefer the term 'gas film' because although leaves of some species retain a gas layer on both sides (i.e. enveloped in gas), others retain a gas layer on only one side due to differences in hydrophobicity between adaxial and abaxial surfaces (Colmer and Pedersen

2008; Winkel *et al.* 2011). Gas films on leaves have been observed in field situations for several terrestrial wetland species when submerged in lakes, ponds, river edges and rice fields on floodplains: rice (Setter *et al.* 1987); *P. australis*, cover of *New Phytologist*, Volume 177(4); *Spartina anglica* (Winkel *et al.* 2011); and own observations (A. Winkel, T. D. Colmer and O. Pedersen). Information on the persistence of gas films on leaves with time following submergence is scant; gas films remained for at least 2 weeks (i.e. evaluation was terminated at 2 weeks) on leaves of *Phalaris arundinacea*, *P. australis* and *Typha latifolia* (all with gas films on both sides) and *Glyceria maxima* (gas film on only the adaxial side) in a controlled environment (Colmer and Pedersen 2008), but for some other species gas films diminish within a few days (own unpublished data; A. Winkel, T. D. Colmer and O. Pedersen).

Gas films on submerged leaves enhance $CO_2$ fixation, as first demonstrated for rice (9- to 10-fold increase; Raskin and Kende 1983). The beneficial effect of leaf gas films to underwater $P_N$ has also been shown for other terrestrial wetland species; at 50 µM dissolved $CO_2$, gas films increased underwater $P_N$ by 1.5- to 6-fold in leaves of four wetland species (Colmer and Pedersen 2008). Data demonstrating the beneficial effect of leaf gas films on underwater $P_N$ are shown for several species in Fig. 3. Apparent resistance to $CO_2$ entry, at environmentally relevant $CO_2$ concentrations in the submergence water, was ~5-fold less in leaves with gas films compared with those with gas films removed (rice and *P. australis*; Pedersen *et al.* 2009).

Leaf gas films provide an enlarged gas–water interface to promote gas exchange with the surrounding floodwater ($CO_2$ uptake during light periods; $O_2$ uptake during dark periods) (Colmer and Pedersen 2008; Pedersen *et al.* 2009). In addition to the enlarged gas–water interface, leaf gas films might also enable stomata to remain open when leaves are submerged. By contrast, for leaves without gas films, stomata are hypothesized to close upon submergence (Mommer and Visser 2005), so that $CO_2$ and $O_2$ must then transverse the cuticle (Mommer *et al.* 2004). The beneficial effect of leaf gas films on underwater $P_N$ was not only demonstrated by the marked decreases when these were removed (Fig. 3), but also leaves with this feature had higher rates of underwater $P_N$ than leaves from species without leaf gas films (Fig. 3). Thus, leaf gas films appear to enable rates of underwater $P_N$ by terrestrial leaves similar to those achieved by submergence-acclimated leaves of terrestrial wetland plants (data and discussion in Colmer and Pedersen 2008). Terrestrial species possessing leaf gas films would benefit from enhanced underwater $P_N$ during short to medium periods of submergence, depending on persistence of the films. By contrast, for species lacking leaf gas films but that produce new acclimated leaves under water, these new leaves take several days to produce so that $P_N$ would likely be less during the initial submergence period, but continued production of acclimated leaves would benefit these species during medium to prolonged submergence.

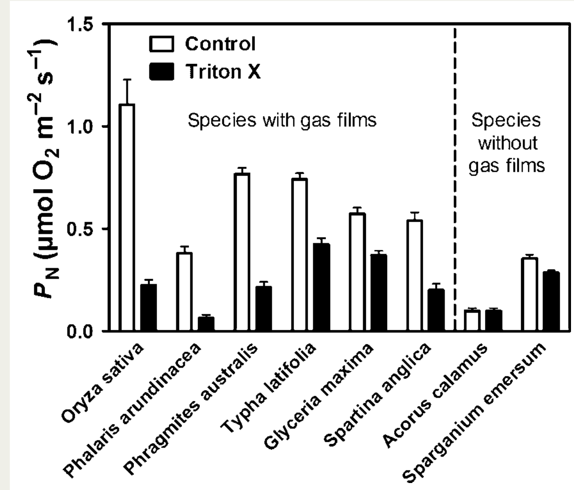


**Fig. 3 Underwater net photosynthesis in terrestrial wetland plants with or without leaf gas films and when gas films were removed.** Measurements for six species were conducted with 50 µM $CO_2$ at 20 °C and photosynthetically active radiation (PAR) of 600 µmol m$^{-2}$ s$^{-1}$; the exceptions were *Oryza sativa* (30 °C; PAR 350 µmol m$^{-2}$ s$^{-1}$) and *S. anglica* (15 µM $CO_2$; PAR 550 µmol m$^{-2}$ s$^{-1}$). These reflect the higher temperatures in tropical rice fields (*O. sativa*) and the lower $CO_2$ in seawater that submerges *Spartina* marshes. Gas films were removed from leaf surfaces by brushing with 0.05% Triton X-100. Species lacking leaf gas films were also brushed with Triton X-100 and showed no, or only a slight, reduction in $P_N$. Data from Colmer and Pedersen (2008), Pedersen *et al.* (2009) and Winkel *et al.* (2011).

Detailed knowledge on leaf gas films is available only for rice (one cultivar only; Pedersen *et al.* 2009). Measurements using $O_2$ microelectrode profiling determined that gas film thickness varied from <10 to 140 µm; positional differences mainly resulted from ridges on leaves (i.e. gas films thinner at the tops of ridges, thicker between adjacent ridges). Using a 'buoyancy method' to measure gas volumes on the surfaces, and within, submerged leaves, showed that tissue porosity was 19% (v/v) and the gas volume of the films was 3.8 times more than the gas within the rice leaf. Diffusive boundary layer widths adjacent to submerged leaves with gas films were surprisingly larger than those adjacent to submerged leaves without gas films, so the enlarged water–gas interface provided by the gas films would have been the major mechanism that reduced resistance to gas exchange of the leaves when under water. At dissolved $CO_2$ concentrations of relevance to field conditions (15–180 μM; e.g. in Thailand, Setter *et al.* 1987; India, Ram *et al.* 1999), underwater $P_N$ was enhanced 4- to 4.9-fold by gas films on leaves of rice (Pedersen *et al.* 2009). Underwater $P_N$ by leaves with gas films and $CO_2$ at near-ambient concentrations was 22% of $P_N$ in air. When gas films were removed artificially from leaves of completely submerged rice, tissue sugar levels and growth were both reduced. Thus, leaf gas films contribute to submergence tolerance of rice by enhancing $CO_2$ entry for underwater $P_N$.

The experiments by Pedersen *et al.* (2009) also elucidated that when rice leaves are in flowing water (15 mm s$^{-1}$; simulating low flows such as might occur across rice fields), the gas film oscillates and the transition zone between mass flow in the bulk medium and diffusion in the boundary layer was wider, and more variable, than for leaves without a gas film. Oscillations of leaf gas films in flowing water were also noted by Barthlott *et al.* (2010), and they reported that specialized surface hairs on the leaves of *Salvinia molesta* can stabilize the gas film, even in fast-flowing water (such as in streams). The leaf surface of *S. molesta* possesses 'eggbeater-shaped hairs' that are hydrophobic except for the tips, a feature that enables gas film formation and retention by 'pinning' the water–air interface (Barthlott *et al.* 2010). The presence of this feature was suggested to prevent the formation and detachment of bubbles that otherwise could occur when in fast-flowing waters (Barthlott *et al.* 2010). This is a very interesting leaf surface feature, although the ecophysiological significance could be debated as *S. molesta* is a floating plant not typically found in fast-flowing waters; the large gas volume trapped by these specialized structures on the surface of the leaves would contribute significantly to the buoyancy of this floating plant.

In addition to enhanced $CO_2$ uptake for photosynthesis, leaf gas films also improve $O_2$ uptake during darkness from floodwaters into leaves (Colmer and Pedersen 2008; Pedersen *et al.* 2009). Thus, leaf gas films enhance leaf $O_2$ status both during the daytime and during nights, with benefits also of improved internal aeration of the entire body of submerged plants. Oxygen derived from $P_N$ during light periods, as well as $O_2$ entry from the floodwater into leaves when in darkness, moves internally via aerenchyma to roots of rice (Pedersen *et al.* 2009) and rhizomes and roots of *S. anglica* (Winkel *et al.* 2011).

In conclusion, our recent studies of leaf gas films (Colmer and Pedersen 2008; Pedersen *et al.* 2009; Winkel *et al.* 2011) have supported the hypothesis by Setter *et al.* (1989), who observed this feature on submerged rice in field situations in Thailand, that gas films provide 'an interface between the gas and water phases for collection of $CO_2$ and dispersal of $O_2$ during the day or collection of $O_2$ during the night'. This mechanism is analogous to the gas layer (plastron) on some aquatic insects that provides an enlarged gas–water interface between the tracheary system and surrounding water (Thorpe and Crisp 1949; Raven 2008; Pedersen and Colmer 2012). For terrestrial wetland species, the few data available indicate that leaf gas films enable rates of underwater $P_N$ similar to those achieved by submergence-acclimated leaves, in both cases being higher than in terrestrial air-formed leaves without these features (data and discussion in Colmer and Pedersen 2008).

## Conclusions and future perspectives

Submergence can have adverse effects on terrestrial wetland plants because of restricted gas exchange and low light. Floodwaters are variable in dissolved $O_2$, $CO_2$, light and temperature. Few data are available on key environmental parameters in various submergence environments—yet these factors influence underwater $P_N$, plant growth and survival. Knowledge of floodwater conditions will enhance one's understanding of plant performance during submergence and enable the design of controlled experiments that better simulate particular submergence environments.

Submergence tolerance of terrestrial wetland plants is influenced by leaf traits. Although terrestrial wetland plants generally lack the numerous beneficial leaf traits possessed by aquatic plants, the few studies available demonstrate that some terrestrial species produce new leaves with a thinner cuticle under water and others possess leaf gas films. The improved gas diffusion between leaves and floodwaters enhances underwater $P_N$ and so contributes significantly to sugar and $O_2$ supply of submerged plants. However, studies of leaf gas film functioning are in their infancy. Our priorities are (i) quantification of the occurrence and persistence of leaf gas films amongst a wide number of wetland species, and determination of whether this trait is related to species distributions in various flood-prone wetlands (cf. analysis of shoot elongation trait; Voesenek *et al.* 2004) and (ii) evaluation of whether rice, or its relatives, possesses variation in leaf gas film formation and persistence, and elucidation of the underlying genetic control of this trait using the array of resources available in rice.

More broadly, there are surprisingly few studies on $P_N$ by terrestrial wetland plants when emergent and when submerged. Also lacking are measurements of $P_N$ with time after submergence and de-submergence. Future studies should compare the performances of species from various habitats, using a range of appropriate bases of expression of $P_N$ rates (area, mass, chlorophyll and leaf N) to facilitate interdisciplinary comparisons by aquatic and terrestrial plant biologists.

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## Contributions by the authors

A.W. compiled the literature and drafted the section 'The submergence environment during overland floods'. T.D.C. and O.P. contributed equally to the remaining sections.

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## Conflicts of interest statement

None declared.

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## Appendix 1: List of wetland plant species grouped into four types: terrestrial, amphibious homophyllous, amphibious heterophyllous and submerged aquatic

These species were used as data were available on underwater net photosynthesis ($P_N$) and specific leaf area (SLA) (see body of table for sources of data).

| | Sources of information | | |
|---|---|---|---|
| | Underwater $P_N$ (used in Fig. 1) | SLA (used in Figs 1 and 2) | $P_N$ in air (used in text) |
| Terrestrial $n = 10$ | | | |
| *Equisetum palustre* | Sand-Jensen *et al.* (1992) | Andersson and Lundegård (1999) | Data not available |
| *Phragmites australis* | Sand-Jensen *et al.* (1992) | Colmer and Pedersen (2008) | Hellings and Gallagher (1992) |
| *Epilobium hirsutum* | Sand-Jensen *et al.* (1992) | Nielsen (1993) | Data not available |
| *Carex elata* | Sand-Jensen *et al.* (1992) | Meziane and Shipley (2001) | Busch and Lösch (1998) |
| *Poa pratensis* | Sand-Jensen *et al.* (1992) | Meziane and Shipley (2001) | Data not available |
| *Chrysosplenium alterniflorum* | Sand-Jensen *et al.* (1992) | Wang *et al.* 2009 | Data not available |
| *Ranunculus repens* | Sand-Jensen *et al.* (1992) | Nielsen (1993) | Lynn and Waldren (2002) |
| *Solanum dulcamara* | Sand-Jensen *et al.* (1992) | Flynn *et al.* (2006) | Data not available |
| *Barbarea stricta* | Sand-Jensen *et al.* (1992) | Nielsen (1993) | Data not available |
| *Cardamine amara* | Sand-Jensen *et al.* (1992) | Nielsen (1993) | Data not available |
| Amphibious (homophyllous) $n = 7$ | | | |
| *Catabrosa aquatica* | Sand-Jensen *et al.* (1992) | Nielsen (1993) | Not considered |
| *Glyceria maxima* | Sand-Jensen *et al.* (1992) | Colmer and Pedersen (2008) | Not considered |
| *Myosotis laxa* | Sand-Jensen *et al.* (1992) | Lenssen *et al.* (2003) | Not considered |
| *Veronica anagallis* | Sand-Jensen *et al.* (1992) | Nielsen (1993) | Not considered |
| *Veronica beccabunga* | Sand-Jensen *et al.* (1992) | Nielsen (1993) | Not considered |
| *Berula erecta* | Sand-Jensen *et al.* (1992) | Nielsen (1993) | Not considered |
| *Myosotis palustris* | Sand-Jensen *et al.* (1992) | Nielsen (1993) | Not considered |
| Amphibious (heterophyllous) $n = 5$ | | | |
| *Callitriche cophocarpa* | Sand-Jensen *et al.* (1992) | Nielsen (1993) | Not considered |
| *Callitriche stagnalis* | Sand-Jensen *et al.* (1992) | Tom Vindbæk Madsen, personal communication | Not considered |
| *Sparganium emersum* | Sand-Jensen *et al.* (1992) | Colmer and Pedersen (2008) | Not considered |
| *Sparganium erectum* | Sand-Jensen *et al.* (1992) | Nielsen and Sand-Jensen (1989) | Not considered |
| *Sagittaria sagittifolia* | Sand-Jensen *et al.* (1992) | Dina Ronzhina, personal communication | Not considered |
| Aquatic $n = 7$ | | | |
| *Lemna trisulca* | Sand-Jensen *et al.* (1992) | Dina Ronzhina, personal communication | Not considered |
| *Potamogeton perfoliatus* | Sand-Jensen *et al.* (1992) | Spence *et al.* (1973) | Not considered |
| *Elodea canadensis* | Sand-Jensen *et al.* (1992) | Madsen *et al.* (1996) | Not considered |
| *Potamogeton crispus* | Sand-Jensen *et al.* (1992) | Nielsen and Sand-Jensen (1989) | Not considered |

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| | Sources of information | | |
|---|---|---|---|
| | Underwater $P_N$ (used in Fig. 1) | SLA (used in Figs 1 and 2) | $P_N$ in air (used in text) |
| *Potamogeton pectinatus* | Sand-Jensen *et al.* (1992) | Nielsen and Sand-Jensen (1989) | Not considered |
| *Batrachium peltatum* | Sand-Jensen *et al.* (1992) | Nielsen (1993) | Not considered |
| *Batrachium aquatile* | Sand-Jensen *et al.* (1992) | Nielsen and Sand-Jensen (1989) | Not considered |

## Appendix 2: List of several studies of underwater net photosynthesis ($P_N$) in terrestrial wetland plants or amphibious plants, in addition to Sand-Jensen *et al.* (1992) (see Appendix 1)

Species, $CO_2$ concentrations, temperatures and light (PAR) regimes used for measurements of underwater $P_N$ are listed. For multi-species studies of underwater $P_N$ in submerged aquatic plants, see Sand-Jensen (1989), Reiskind *et al.* (1989) and Madsen *et al.* (1993*b*).

| Source | Species tested | $CO_2$ (μM) | Temperature (°C) | PAR (μmol m$^{-2}$ s$^{-1}$) | Notes |
|---|---|---|---|---|---|
| Nielsen (1993) | *Barbarea stricta, Batrachium aquatile, Berula erecta, Callitriche cophocarpa, Cardamine amara, Catabrosa aquatile, Epilobium hirsutum, Glyceria maxima, Hydrocotyle vulgaris, Littorella uniflora, Lobelia dortmanna, Lotus uliginosus, Montia fontana, Myosotis palustris, Polygonum amphibium, Ranunculus repens, Sparganium emersum, Sparganium erectum, Veronica anagallis-aquatica, Veronica beccabunga* | 100 | 25 | 600 | Also $P_N$ rates in air, although some seem unusually high |
| Sand-Jensen and Frost-Christensen (1998) | *Myosotis palustris, Sparganium emersum* | 20 and 280 | 12 and 24 | 400 | Also effects of initial $O_2$ concentration and temperature on underwater $P_N$ |
| Sand-Jensen and Frost-Christensen (1999) | *Berula erecta, Menta aquatica, Myosotis palustris, Veronica anagallis-aquatica* | 100 and 700 | 15 | 350 | Also initial slope at $P_N$ rate-limited $CO_2$ concentrations and $P_{max}$ |
| Vervuren *et al.* (1999) | *Arrhenatherum elatius, Phalaris arundinacea, Rumex crispus* | 2,200 | 20 | 740 | Also $P_N$ rates under water after 30 days of submergence |
| Nielsen and Nielsen (2006) | *Lobelia cardinalis, Nesaea crassicaulis* | 40 and 1500 | 20 | 1200 | Also $P_N$ rates in air |
| Mommer *et al.* (2007) | *Rumex palustris* | 10–10 000 | 20 | 400 | Also full $CO_2$ response curve and $P_N$ rate in air |
| Pedersen *et al.* (2006) | *Halosarcia pergranulata* (syn. *Tecticornia pergranulata*) | 20–6800 | 20 | 1500 | Also $P_N$ rate in air |

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***Continued***

| Source | Species tested | $CO_2$ (μM) | Temperature (°C) | PAR (μmol $m^{-2}$ $s^{-1}$) | Notes |
|---|---|---|---|---|---|
| Colmer and Pedersen (2008) | *Acorus calamus, Glyceria maxima, Phalaris arundinacea, Phragmites australis, Sparganium emersum, Typha latifolia* | 50 and 500 | 20 | 600 | Also full $CO_2$ response curve for *Phragmites australis* |
| Pedersen *et al.* (2009) | *Oryza sativa* | 15–2000 | 30 | 350 | Also full $CO_2$ response curve under water and $P_N$ rate in air |
| Pedersen *et al.* (2010) | *Hordeum marinum* | 18–2000 | 20 | 350 | Also full $CO_2$ response curve under water and $P_N$ rate in air |